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12 Cysteine Homeostasis

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12.1 Abstract

In plant metabolism, cysteine is the first reduced sulfur donor molecule, and it constitutes the metabolic precursor of essential biomolecules, such as vitamins, cofactors, antioxidants and many defence compounds, that are formed in response to adverse environmental conditions in which the sulfur moiety is the functional group. Sulfate is transported inside the plant, reduced to sulfide and incorporated into *O*-acetylserine (OAS) to form cysteine. The enzymes used in the last step of cysteine biosynthesis, serine acetyltransferase (SAT, also called SERAT) and *O*-acetylserine(thiol)lyase (OASTL), form a complex that senses the sulfur nutrient status of the plant and modulates cysteine biosynthesis.

Arabidopsis thaliana contains different OASTL and SAT enzymes in the different cellular compartments, resulting in different sub-cellular pools of cysteine. In recent years, significant progress has been made in determining the specific roles of the different sub-cellular pools of cysteine, as well as its associated metabolites. In chloroplasts, OAS can serve either as the substrate of the OASTL isoform OAS-B in the stroma to produce cysteine, or as the substrate of the OASTL isoform CS26 in the thylakoid lumen, which uses thiosulfate instead of sulfide to produce *S*-sulfocysteine. *S*-sulfocysteine is a signalling molecule that is involved in the regulation and protection of the photosystems of the plant. In the mitochondria, cysteine is essential for detoxifying cyanide by a loop that is established by the activities of the OASTL isoforms CYS-C1 and OAS-C. Mitochondrial cyanide regulates the root hair development and modulates the plant immune response. The main OASTL isoform, OAS-A1, and DES1, an OASTL isoform that acts as a cytosolic desulfhydrase and degrades cysteine, contribute to the homeostasis of the cytosolic cysteine and sulfur. Cytosolic sulfide acts as a signalling molecule, regulating the process of autophagy, while cytosolic cysteine is essential for inducing the hypersensitive response to pathogens. Thus, cytosolic cysteine homeostasis contributes to orchestrating the plant response to pathogens.

12.2 Introduction

Sulfur is an essential macronutrient that is present in nature in organic and inorganic forms. In its different oxidation states, it represents one of the most versatile elements, and the most common oxidized form is sulfate (SO_4^{2-}), which contains an S atom with a redox state of +6. Plants, microorganisms and fungi, differently from animals, are able to reduce sulfate to sulfide and to incorporate sulfur into organic metabolites through an energy-dependent process, thereby originating a large variety of organic compounds that are essential for plant

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growth and development. Plants are the most important source of sulfur-containing amino acids (mainly methionine and cysteine) for humans and animals (Wirtz and Droux, 2005). Sulfur is present in vitamins such as biotin and thiamine, cofactors such as *S*-adenosylmethionine (SAM), coenzyme A, molybdenum cofactor (CoMo), and lipoic acid and iron–sulfur clusters that participate in electron transport. Glucosinolates, phytoalexins, thionines and defensins are sulfur-containing compounds that are involved in the plant response to pathogens (Rausch and Wachter, 2005). Sulfur is also present in sulfolipids, which are essential for the formation of thylakoid membranes in the chloroplasts. Some plant hormones are inactivated by sulfatation, including brassinosteroids and jasmonic acid; in contrast, sulfatation is essential for the function of peptide hormones that stimulate cellular growth (Hirai and Saito, 2008). Sulfur compounds are also of great importance for food quality and the production of phyto- pharmaceuticals (Moriarty *et al*., 2007). Sulfur deficiency will result in the loss of plant production, fitness and resistance to environmental stress and pests.

Cysteine is the first reduced sulfur organic compound that is synthesized from the plant photosynthetic assimilation of sulfate. Cysteine is essential not only as a proteinogenic amino acid, but also for stabilizing the tertiary and quaternary structures of proteins, because it participates in the formation of disulfide bonds and regulates the active sites of many enzymes. Cysteine plays a central role in the primary and secondary metabolism of the plant owing to its biochemical functions. It is the precursor metabolite of the antioxidant glutathione, the amino acid methionine and most of the essential biomolecules and above-mentioned defence compounds; the catalytic mechanism of many of these compounds is based on the reactivity of the thiol group (Droux, 2004). The conversion between free thiol groups and disulfide bond formation is a dynamic system that is the basis for protein redox modifications. These changes are involved in regulating the activity of certain enzymes that, in turn, regulate cellular metabolism in response to environmental cues (Wirtz and Droux, 2005).

12.3 Photosynthetic Assimilation of Sulfate in Plants

The use of bacteria and yeast mutants, as well as the complete genome sequence of *Arabidopsis thaliana*, has led to a breakthrough in the understanding of the sulfate assimilation process in plants (Barroso *et al*., 1997; Hell 1997; Leustek *et al*., 2000; Noji *et al*., 2006; Saito 2004; Takahashi *et al*., 2011). The biosynthesis of cysteine is the last step of the photosynthetic sulfate assimilation. The three basic steps of the process are as follows: sulfate transport into the cell, the reduction of sulfate to sulfide and the incorporation of the reduced sulfur into the carbon skeletons (Fig. 12.1).

12.3.1 Sulfate transport

Plants take sulfate up from the soil via their roots and it is then distributed to the entire plant. At the root level, sulfate is transported through the plasma membrane by a proton/sulfate co-transport mechanism. It is subsequently loaded into the xylem vessels and transported to the shoot by the transpiration stream using the energy that is driven by a proton ATPase-generated proton gradient. The uptake of sulfate by the roots is strictly controlled and appears to be the most regulated step in the plant sulfur assimilation pathway (Vauclare *et al*., 2002).

The sulfate transporters, which are proteins, all possess 12 transmembrane domains in the central zone of the polypeptide. The differences in the amino acid sequences of the amino and carboxy terminal ends of the proteins determine the differences between the proteins in their affinity for sulfate and their specific functions (Vidmar *et al*., 2000). The sulfate transporters also contain in their carboxy terminus a domain called STAS (sulfate transporters and anti-sigma factor antagonists), which may play a regulatory role in controlling the activity and localization of membrane transporters (Shibagaki and Grossman, 2004; Rouached *et al*., 2005). In *Arabidopsis thaliana*, 14 genes have been identified that form a

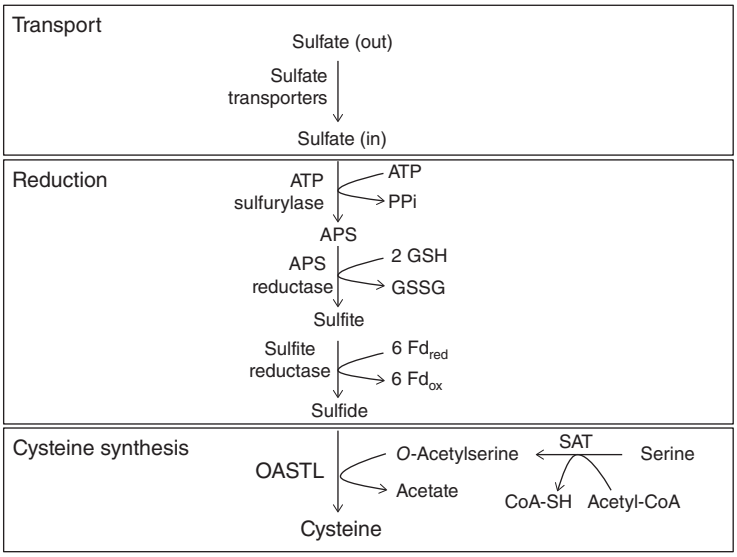

Fig. 12.1. Sulfate assimilation in plants. APS, adenosine 5′-phosphosulfate; Fd, ferredoxin; GSH, reduced glutathione; GSSG, oxidized glutathione; OASTL, *O*-acetylserine(thiol)lyase; PPi, pyrophosphate; SAT, serine acetyltransferase.

family of sulfate transporters (Howarth *et al.*, 2003b). According to their sequences, these genes can be divided into five groups (SULTR1–SULTR5), depending on both the substrate affinity of the protein that is produced and the function that it exerts (Hawkesford and De Kok, 2006).

12.3.2 Sulfate reduction

In the roots or shoots, sulfate can be stored in the vacuoles, or it can be transported to the shoot chloroplasts, where it is reduced. The intracellular distribution of sulfate is regulated by the available concentrations of cysteine or glutathione (Bolchi *et al.*, 1999).

Sulfate must be activated to adenosine 5′-phosphosulfate (APS) before it is reduced. ATP sulfurylases (EC 2.7.7.4) catalyse the addition of sulfate to the α-phosphate of ATP. In *Arabidopsis*, four genes code for the ATP sulfurylases, three of which are plastidial and one cytosolic (Hatzfeld *et al.*, 2000; Mugford *et al.*, 2009). All of these genes are expressed in the shoots and the roots (Lee and Leustek 1999), even though sulfate activation and reduction take place predominantly in the leaf chloroplast.

APS is reduced to sulfite by APS reductase (APR; EC 1.8.99.2) using glutathione as a reductant. *Arabidopsis* has three genes coding for APR, which encode polypeptides of 50 kDa presenting an amino-terminal catalytic domain that is homologous to the bacterial APS and 3′-phosphoadenosine-5′-phosphosulfate (PAPS) reductases and a carboxy-terminal domain that interacts with the reductant homologue of thioredoxins and glutaredoxins (Gutierrez-Marcos *et al.*, 1996; Setya *et al.*, 1996). The reduction of APS is the key regulation point in sulfate assimilation, and APR activity is highly regulated according to the demand for reduced sulfur (Lee *et al.*, 2011).

Sulfite is reduced to sulfide by sulfite reductases (EC 1.8.7.1) which have a high affinity for the ferredoxin that they use as a reductant (Yonekura-Sakakibara *et al.*, 2000). Sulfite reductases are homo-oligomeric haemoproteins with two to four identical subunits. *Arabidopsis* has a single gene coding for this activity.

12.3.3 Cysteine biosynthesis

The synthesis of cysteine in plants represents the final step of assimilatory sulfate reduction and the almost exclusive entry mechanism for reduced sulfur into the metabolism of not only plants but also into the human food chain in general. In plants, cysteine is synthesized in the cytosol, plastids and mitochondria by the sequential action of the enzyme serine acetyltransferase (SAT, also known as SERAT; EC 2.3.1.30), which synthesizes the intermediary product, *O*-acetylserine (OAS), and *O*-acetylserine(thiol) lyase (OASTL; EC 2.5.1.47), which combines a sulfide molecule with an OAS molecule to produce L-cysteine. The formation of cysteine is the direct coupling step between sulfur and nitrogen assimilation in plants.

In *A. thaliana*, nine OASTL- and five SAT-encoding genes have been identified by sequence homology (Table 12.1), and the functionality of some of these genes has been demonstrated by several studies.

A phylogenetic analysis of the SATs indicates that the isoforms SAT106 and SAT-N, which are located in the cytosol, are very closely related, as are the plastidial SAT53 and the mitochondrial SAT1 SAT isoforms. The cytosolic SAT52 isoform is the most divergent SAT, but it is more closely related to the cytosolic than to the organellar SATs (Fig. 12.2A).

The phylogenetic tree of the OASTLs shows that chloroplastic isoform OAS-B and the mitochondrial OAS-C are very closely related, that the cytosolic isoforms DES1, CYS-D1 and CYS-D2 diverged recently and that the mitochondrial CYS-C1 isoform is the most divergent protein of the family (Fig. 12.2B).

12.4 The Cysteine Synthase Complex: Regulation of Cysteine Biosynthesis

It is well established that SAT and OASTL physically interact to form the hetero-oligomeric cysteine synthase complex (CSC), which is composed by a dimer of SAT trimers that are associated with two homodimers of OASTL ($\alpha_6\beta_4$). The SAT enzymes possess two conserved domains: an N-terminal domain that is rich in α-helix structures that are involved in SAT/SAT interaction and a β-sheet-containing the C-terminal domain that is responsible for the SAT/OASTL interactions, as well as for catalysis (Wirtz and Hell, 2006). OASTL forms stable homodimers with a molecular weight of 68–75 kDa that carry two molecules of pyridoxal-5′-phosphate (PLP) per dimer as a prosthetic group (León *et al*., 1987). The CSC is a part of the regulatory circuit controlling sulfur homeostasis (Fig. 12.3). While OASTL is only active outside the CSC,

Table 12.1. Serine acetyltransferase (SAT) and *O*-acetylserine(thiol)lyase (OASTL) families in *Arabidopsis thaliana*.

Protein	Gene ID	Subcellular location	References
SATs			
SAT1	At3g13110	Mitochondria	Krueger *et al*., 2009
SAT106	At2g17640	Cytosol	Howarth *et al*., 2003a)
SAT52	At5g56760	Cytosol	Krueger *et al*., 2009
SAT53	At1g55920	Plastids	Krueger *et al*., 2009
SAT-N	At4g35640	Cytosol	Howarth *et al*., 2003b
OASTLs			
Atcys-C1	At3g61440	Mitochondria	García *et al*., 2010
Atcys-D1	At3g04940	Cytosol	Jost *et al*., 2000
Atcys-D2	At5g28020	Cytosol	Jost *et al*., 2000
CS26	At3g03630	Plastids	Bermúdez *et al*., 2010
DES1	At5g28030	Cytosol	Álvarez *et al*., 2010
OAS-A1	At4g14880	Cytosol	Barroso *et al*., 1995
OAS-A2	At3g22460	None	Jost *et al*., 2000
OAS-B	At2g43750	Plastids	Heeg *et al*., 2008
OAS-C	At3g59760	Mitochondria	Álvarez *et al*., 2012c

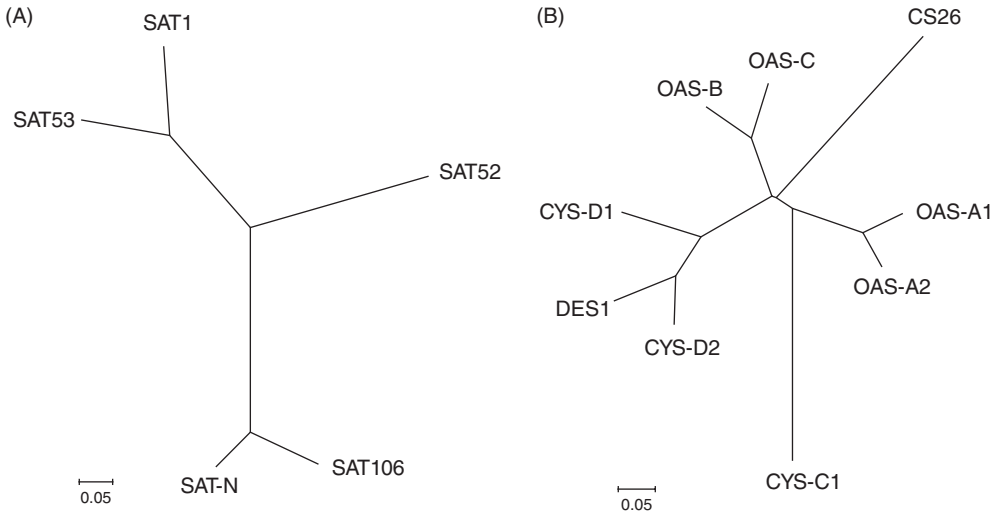

Fig. 12.2. The phylogeny of (A) serine acetyltransferase (SATs) and(B) *O*-acetylserine(thiol)lyases (OASTLs) in *Arabidopsis thaliana*. The phylogenetic analyses were conducted using the Molecular Evolutionary Genetics Analysis software version 5 (MEGA5) (Tamura *et al*., 2011). The complete protein sequences were aligned using MUSCLE software and the unrooted phylogenetic tree was inferred by the neighbour-joining method; all of the methods were integrated in the MEGA tool.

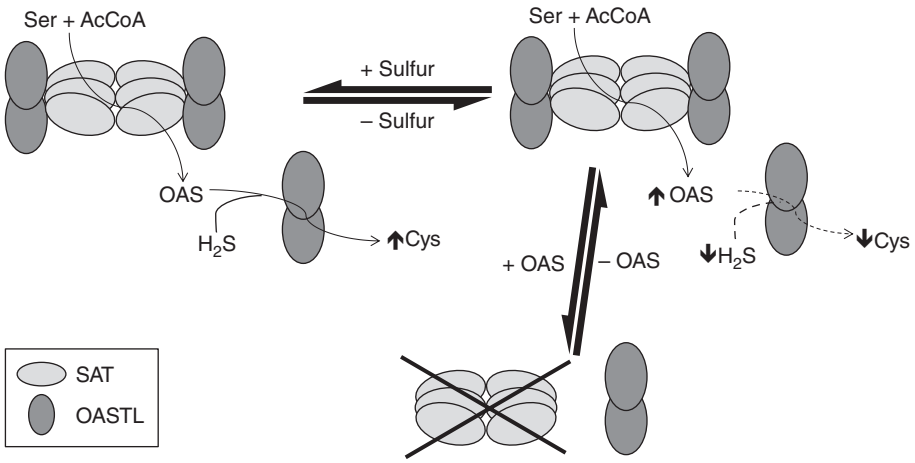

Fig. 12.3. Regulation of the cysteine synthase complex (CSC). AcCoA, acetyl-CoA; Cys, cysteine; OAS, *O*-acetylserine; OASTL, *O*-acetylserine(thiol)lyase; SAT, serine acetyltransferase Ser, serine.

SAT activity requires an association with OASTL (Droux *et al*., 1998). The CSC is stabilized by free sulfide and destabilized by OAS. Furthermore, OASTL activity exceeds SAT activity by 345 times in plastids, 200 times in the cytosol and ten times in the mitochondria, indicating that the total SAT is found in the CSC together with a small part of OASTL (Heeg *et al*., 2008). So when sulfur is not limiting, the sulfide that is produced in the chloroplasts by the sulfate assimilation pathway stabilizes the CSC, which produces OAS that combines with sulfide via the free OASTL. When OAS is in excess, the CSC dissociates, and the SAT activity decreases drastically. The CSC, then, effectively senses both the OAS and the sulfur availability and

self-regulates further OAS production according to the supply and demand.

In addition to its transcriptional regulation and regulation by the CSC, the cytosolic SAT isoform is also retro-inhibited by cysteine. This feature is not observed in the chloroplastic and mitochondrial isoforms, which are insensitive to cysteine levels (Noji *et al.*, 1998; Kumaran *et al.*, 2009).

12.5 Cysteine Synthesis in Cellular Compartments

The fact that the assimilatory reduction of sulfate takes place in the chloroplast suggests that the major plastidial OASTLs and SATs participate in the primary assimilation pathway. The other isoforms (those in a minority or located in organelles other than the chloroplasts) would then exhibit other metabolic and/or signalling functions. Our research group is interested in unravelling the function of each subcellular pool of cysteine. The detailed study of *Arabidopsis* T-DNA insertional mutants in each of these isoforms, the application of '-omics' technologies and the identification of the distribution of the cellular enzymatic activities and metabolites has allowed the determination of the specific functions of the different OASTL and SAT isoforms and, therefore, has also allowed the contribution of the different subcellular pools of cysteine to plant physiology to be determined.

Initially, it was supposed that cysteine does not permeate membranes owing to its thiol group reactivity and that every compartment that is able to synthesize proteins would need its own cysteine production (Lunn *et al.*, 1990). The lack of plastidial cysteine biosynthesis in the mutant of the major chloroplastic isoform OAS-B is completely compensated for by the cysteine that is synthesized in the other compartments; as a result, a membrane transport of sulfide from the chloroplast to the other cellular compartments is required (Heeg *et al.*, 2008). It has been speculated that sulfide reaches the cytosol by diffusion through the chloroplast membranes. Hydrogen sulfide (H_2S) is a lipophilic molecule that freely permeates plasma membranes, although its ionized forms, HS^- and S^{2-}, cannot permeate membranes (Kabil and Banerjee, 2010). H_2S is weakly acidic and dissociates in aqueous solutions. Under physiological pH conditions (pH 7.4), one third of the H_2S that is present is not dissociated, and the remaining two thirds dissociate into H^+ and HS^-. Under high pH conditions, HS^- dissociates into H^+ and S^{2-}, which cannot permeate membranes. So at the pH of 8.5 that is maintained by the chloroplast stroma under illumination, sulfide is mainly present in its charged form and, consequently, is unable to undergo transport across the chloroplast envelope. Therefore, sulfide needs to be transported actively across the chloroplast membrane (Heldt *et al.*, 1973; Wu and Berkowitz 1992; Romero *et al.*, 2013). Additionally, the analysis of SAT-deficient mutants has demonstrated that the mitochondria are the main source of OAS in the cell, as they contain 80% of the total SAT activity and show the lowest OASTL activity of all of the organelles (Haas *et al.*, 2008; Krueger *et al.*, 2009). Finally, as demonstrated by mutants that are deficient in the major OASTLs, most of the cysteine is synthesized in the cytosol by the main cytosolic isoform, OAS-A1. OAS-A1 constitutes 23% of the total OASTL proteins and 44 and 80% of the total OASTL activity in the leaves and roots, respectively (Heeg *et al.*, 2008; López-Martín *et al.*, 2008a; Watanabe *et al.*, 2008). The concentration of cysteine in the cytosol has been estimated to be 300 μM, while in other cellular compartments it is below 10 μM (Krueger *et al.*, 2009).

In summary, the analysis of knockout mutants that are deficient in the different major isoforms of OASTL or SAT in the plastids, mitochondria and/or cytosol has revealed that chloroplasts constitute the main source of sulfide, mitochondria are rich is OAS and cysteine is mainly synthesized in the cytosol (see Fig. 12.4). Moreover, the compartments that are not affected by the mutations are able to provide cysteine to the compartment that is affected. As the viability of the plant is not or is only very slightly affected by the mutation of any of the main OASTLs, efficient transport mechanisms of sulfide, OAS and cysteine between the plastids, mitochondria and cytosol should occur.

12.6 Other Members of the *O*-Acetylserine(Thiol)Lyase Gene Family

In *A. thaliana*, the most transcribed *OASTL* genes encode the cytosolic OAS-A1 (At4g14880), the plastidial OAS-B (At2g43750) and the mitochondrial OAS-C (At3g59760) isoforms.

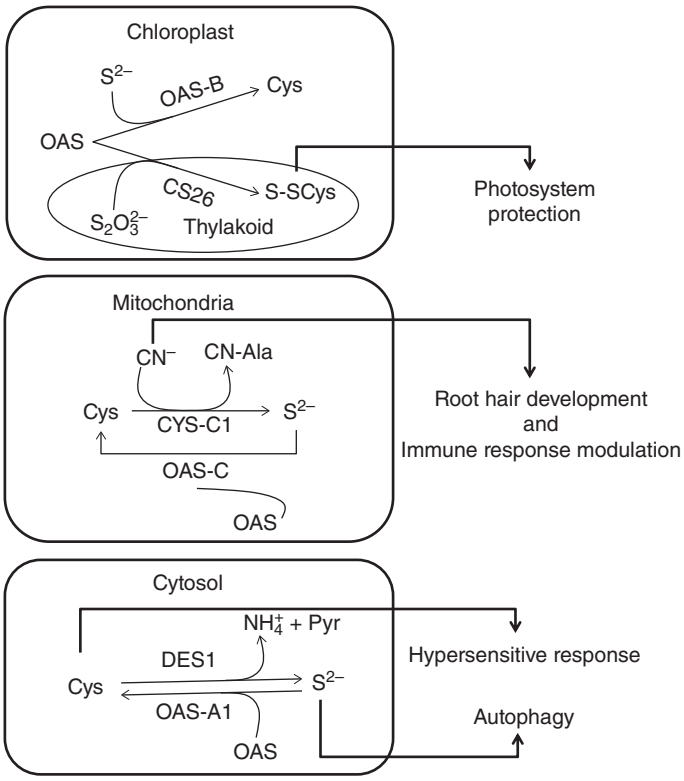

Fig. 12.4. The importance of the compartment-specific pool of cysteine in *Arabidopsis* cells. The function of every pool of cysteine or its associated metabolites is indicated on the right. The most abundant sulfur-associated metabolite in every cellular compartment is indicated with large bold letters. *O*-acetylserine (thiol)lyase (OASTL) isoforms: CS26, CYS-C1, OAS-A1, OAS-B, OAS-C, DES1. Other abbreviations: Cys, cysteine; OAS, *O*-acetylserine; S^{2-}, sulphide; $S_2O_3^{2-}$, thiosulfate

These proteins are considered to be authentic OASTLs because they catalyse the synthesis of cysteine from OAS and sulphide, and they interact with SAT (Bonner *et al.*, 2005; Heeg *et al.*, 2007). One of the other OASTL genes, *OAS-A2*, does not produce a functional protein owing to the presence of an in-frame stop codon and an unspliced intron. In addition to these major OASTLs, the OASTL enzyme family also includes another isoform, CYS-C1, which is located in the mitochondria; this isoform is the most highly expressed isoform, but actually functions as a β-cyanoalanine synthase (Yamaguchi *et al.*, 2000). The remaining OASTL proteins, the cytosolic cys-D1, cys-D2 and DES1, and the plastidic CS26, are expressed at much lower levels, were identified by sequence homology upon the sequencing of the *A. thaliana* genome and were thought to have auxiliary functions with respect to the major isoforms. Our investigations have shed light on to the functions of CS26, CYS-C1 and DES1 as a way to unravel the role of cysteine metabolism in every cellular compartment (see Fig. 12.4).

12.6.1 CS26

In *Arabidopsis* chloroplasts, two OASTL isoforms are present, a true OASTL, OAS-B and CS26, which has *S*-sulfocysteine synthase activity and catalyses the incorporation of thiosulfate into OAS to form *S*-sulfocysteine (S-Cys) (Nakamura *et al.*, 1984; Bermúdez *et al.*, 2010). In photosynthetic organisms, the reductive steps that are catalysed by APS reductase and sulfite reductase are restricted to the plastids; thus, sulfate

reduction occurs only in this compartment (Takahashi *et al*., 2011). Logically, cysteine production via OASTL should be also restricted to the chloroplast; however, OAS-B does not appear to play an essential role in the plant, as plants lacking OAS-B show no apparent phenotype when grown under long- or short-day photoperiods (Bermúdez *et al*., 2012). In contrast, it has been shown that the minor isoform CS26 plays an essential role in chloroplast redox control (Bermúdez *et al*., 2012). CS26 loss-of-function mutants display severe growth inhibition and leaf paleness when grown under long-day photoperiod conditions; this phenotype depends on the photoperiod, as under short-day conditions the *cs26* mutant is indistinguishable from the wild type plants. The long-day specific phenotype is accompanied by an accumulation of reactive oxygen species (ROS), such as superoxide radicals and hydrogen peroxide, in the *cs26* leaves, which is not observed under short-day conditions (Bermúdez *et al*., 2010). The *cs26* mutant plants also show a significant reduction in photosynthetic performance when grown under long-day conditions, manifested as a reduction in the net CO_2 assimilation rate, mesophyll conductance and mitochondrial respiration in darkness, which phenomena are not observed under short-day conditions (Bermúdez *et al*., 2012). The dependence of the *cs26* mutant phenotype on the photoperiod suggests that plants lacking this protein are not able to deal with the light stress that is produced during periods of prolonged light exposure, which damages the photosynthetic machinery and causes the accumulation of ROS and, ultimately, growth inhibition. Therefore, the chloroplastic *S*-sulfocysteine synthase activity is essential for maintaining the balance between ROS production and detoxification in the chloroplast, and also for preventing photochemical damage to the photosystems and to the electron transport chain. In fact, when grown under low light intensity, the *cs26* mutant shows similar photosynthetic parameters to those of plants that are grown under high-light conditions (Bermúdez *et al*., 2012).

OAS-B and CS26 are located in different subcellular compartments and have different enzymatic activities, although they share OAS as a substrate. OAS-B is found in the stroma, has OASTL activity and uses sulfur to incorporate itself into the OAS, whereas CS26 is found in the lumen, where it incorporates thiosulfate into the OAS to produce S-Cys (Bermúdez *et al*., 2010). S-Cys can act as an oxidant, reacting with thiol groups following the reaction:

$$RSSO_3^- \leftrightarrows RSSR + HSO_3^-$$

This allows the oxidation of protein thiol groups in the thylakoid lumen. CS26 would then act as a photosynthesis sensor. Under normal photosynthetic conditions, sulfite is directly converted into sulfide in the chloroplasts. When the photosynthetic machinery is saturated, the ROS accumulate and sulfite is converted into thiosulfate in the chloroplasts (Khan *et al*., 2010). Thiosulfate is detected by the CS26 protein and is converted to S-Cys, which, acting as a mild oxidant in the lumen, triggers protection mechanisms in the photosynthetic apparatus by regulating the activity of enzymes that are involved in photosystem repair or reorganization, such as STN7 (a thylakoid-associated kinase), KBP13 (a thylakoid peptidyl-prolyl isomerase)and Deg1 (a thylakoid protease) (Dietz and Pfannschmidt, 2011; Gotor and Romero, 2013).

12.6.2 CYS-C1

In non-cyanogenic plants, cyanide is a co-product of ethylene biosynthesis. It is also produced during the biosynthesis of camalexin, a phytoalexin formed in *Arabidopsis* plants when they are infected by a large variety of microorganisms, including bacteria, fungi and oomycetes (Glawischnig, 2007). The lethality of the anion cyanide lies in its high reactivity with keto compounds and Schiff base intermediates to give cyanohydrins and stable nitrile derivatives, respectively, and because it chelates divalent and trivalent metal ions in the prosthetic groups of several metalloenzymes. In mitochondria, cyanide binds to the haem iron of cytochrome *c* oxidase, thereby blocking the utilization of oxygen in cellular functions (Donato *et al*., 2007). Hence, cyanide must be rapidly detoxified and metabolized by the plant to keep the concentration below toxic levels. *Arabidopsis* plants carry the mitochondrial β-cyanoalanine synthase (CAS) CYS-C1 (Watanabe *et al*., 2008), which belongs, together with the OASTLs, to the family of β-substituted alanine synthase enzymes. CAS

is a PLP-dependent enzyme that uses cysteine to detoxify cyanide by converting cyanide and cysteine into hydrogen sulfide (H_2S) and β-cyanoalanine, the latter being converted later to asparagine (Asn), aspartate (Asp) and ammonia by NIT4 class nitrilases, thus recycling the nitrogen for the plant (Piotrowski, 2008). H_2S also blocks the mitochondrial respiratory pathway and, for this reason, needs to be detoxified by the true OASTL, OAS-C, which produces cysteine from OAS and H_2S that can be used by CYS-C1 to detoxify cyanide, so completing the cyanide detoxification cycle in mitochondria (Álvarez *et al.*, 2012c).

The *CYS-C1* or *OAS-C* loss-of-function mutations are not toxic for the plant and lead to an increased level of cyanide in *cys-c1* and *oas-c* null mutants as well as a root hairless phenotype, suggesting that cyanide plays a signalling role in root development (García *et al.*, 2010; Álvarez *et al.*, 2012c). In both cases, the genetic complementation of the *cys-c1* and *oas-c* null mutants with the corresponding *CYS-C1* and *OAS-C* gene rescues the root hair elongation impairment, restoring the wild type phenotype and thereby confirming that the observed phenotypes were indeed due to the mutations (García *et al.*, 2010; Álvarez *et al.*, 2012c). Furthermore, the root hair defect is phenocopied in wild type plants by the exogenous addition of cyanide to the growth medium and is reversed by the addition of hydroxocobalamin, which is the most commonly used antidote for severe acute cyanide poisoning in humans (García *et al.*, 2010).

In addition to its role in plant root development, cyanide has been proposed to act as a regulator of other biological processes in plants, such as seed dormancy and germination (Cohn and Hughes, 1986; Bethke *et al.*, 2006; Siegien and Bogatek, 2006), and resistance to viral and fungal pathogens (Chivasa and Carr, 1998; Wong *et al.*, 2002; Seo *et al.*, 2011) using exogenously applied cyanide. The *cys-c1* mutant allows for the investigation of the role of endogenously produced cyanide.

We have definitively observed that *cys-c1* plants present an increased susceptibility to the necrotrophic fungus *Botrytis cinerea* and an increased tolerance to the biotrophic *Pseudomonas syringae* pv. *tomato DC3000* bacterium and the *Beet curly top virus*. The *cys-c1* mutation reduces the respiration rate in the leaves, causes the accumulation of ROS and induces the alternative oxidase *AOX1a* and the pathogenesis-related expression of *PR1*, a protein induced by the salicylic acid-dependent pathway. Interestingly, no patches of dead cells are observed when this mutant is grown under either long- or short-photoperiod conditions. We hypothesize that cyanide, which is transiently accumulated during avirulent bacterial infection and constitutively accumulated in the *cys-c1* mutant, uncouples the respiratory electron chain, which is dependent on cytochrome c oxidase, and that this uncoupling induces the alternative oxidase activity and the accumulation of ROS, which stimulate the salicylic acid-dependent signalling pathway of the plant immune system (García *et al.*, 2013).

Considering these results, we hypothesize that the cysteine mitochondrial pool is essential for maintaining the non-toxic concentrations of mitochondrial cyanide and sulfur to allow their signalling role directly or through the controlled accumulation of ROS in this organelle.

12.6.3 DES1

Cysteine is highly reactive and so it can be a very toxic molecule. Thiols are easily oxidized to form species with sulfur in higher oxidation states that subsequently inhibit enzyme activity; furthermore, cysteine reduces the ferric iron at an exceptional rate and promotes oxidative damage through the Fenton reaction (Jacob *et al.*, 2003; Park and Imlay 2003). Consequently, cysteine homeostasis must be precisely maintained in the cytosol. Cysteine is mainly produced *de novo* in the cytosol by the action of the OAS-A1 protein (Heeg *et al.*, 2008; Watanabe *et al.*, 2008) and can be degraded in the cytosol by the action of L-cysteine desulfhydrase (DES; EC 4.4.1.1), which catalyses the formation of sulfide, ammonia and pyruvate from cysteine in a stoichiometric ratio of 1:1:1. In *A. thaliana*, the only cytosolic desulfhydrase that has been described to date is encoded by *DES1* (At5g28030). Knockout *des1* plants show increased cysteine levels and decreased accumulation of ROS, leading to enhanced antioxidant defences and tolerance to conditions that promote oxidative stress (Álvarez *et al.*, 2010). In contrast, *oas-a1* knockout mutants show decreased cysteine levels and an imbalance between the generation and removal of ROS, leading to an accumulation of ROS and

the induction of programmed cell death (PCD) in patches of cells (López-Martín *et al*., 2008a). This imbalance results in an increased sensitivity of *oas-a1* mutants to oxidative stress and the conditions promoting it (López-Martín *et al*., 2008a). The contrasting phenotypes of *des1* and *oas-a1* mutants suggest that cytosolic cysteine is a determinant of the antioxidative capacity of this cellular compartment; therefore, the coordination of the contrasting enzymatic activities of DES1 and OAS-A1 maintains cytosolic cysteine homeostasis and so should influence the processes that are regulated by the cysteine/redox homeostasis (López-Martín *et al*., 2008b; Gotor *et al*., 2010). The tolerance to heavy metals such as Cd is affected by cytosolic cysteine homeostasis, as *des1* mutants are more tolerant to increased concentrations of Cd (up to 250 μM) than are wild type plants (Álvarez *et al*., 2010), and *oas-a1* mutants are more sensitive to low concentrations of this metal (as low as 75 μM) than are wild type plants. Thus, the cysteine levels are directly correlated with plant resistance to Cd-induced stress. In fact, the overexpression of *oas-a1* in *Arabidopsis* and yeast leads to an enhanced tolerance to heavy metals and salinity, respectively (Dominguez-Solis *et al*., 2001, 2004; Romero *et al*., 2001).

During the plant immune response, redox signalling plays an essential role. One of the earliest responses to pathogen infection is the production of ROS (reviewed in Lamb and Dixon, 1997), which are essential in the establishment of resistance. In response to some pathogens, plants can induce the hypersensitive response (HR), which causes the rapid death of the infected cells, so limiting the spread of biotrophic pathogens (Heath, 2000). The establishment of the HR is dependent on the generation of ROS and contributes to the activation of defence in adjacent cells and to systemic acquired resistance (SAR), which is a broad-spectrum form of disease resistance that is mediated by the action of the hormone salicylic acid (Vlot *et al*., 2008). The study of the response of *des1* and *oas-a1* mutant plants to biotrophic and necrotrophic pathogens has shown that the cytosolic cysteine content is directly correlated with the resistance; therefore, an increase of cytosolic cysteine increases the plant resistance to pathogens, while a decrease of cytosolic cysteine decreases the plant resistance to pathogens (Álvarez *et al*., 2012a). The *des1* mutants behave as constitutive SAR mutants, in which this systemic response is constitutively activated and the mutants show a high level of resistance to biotrophic and necrotrophic pathogens, the accumulation of salicylic acid and the induction of *WRKY54* and *PR1*. Additionally, *oas-a1* mutations enhance the pathogen sensitivity (Álvarez *et al*., 2012a) and impair the HR that is associated with the infection by the bacteria *Pseudomonas syringae* pv. *tomato DC3000 avrRpm1* (Álvarez *et al*., 2012a). These data suggest that cytosolic cysteine homeostasis, which is driven by the enzymatic activities of OAS-A1 and DES1, plays an essential role in regulating the plant response to pathogens.

The *des1* mutants also exhibit premature leaf senescence, along with an increased expression of senescence-associated genes, such as *SAG12*, *NAP* and *PR1*, and the presence of senescence- associated vacuoles (SAVs) (Álvarez *et al*., 2010, 2012b). In contrast, the *oas-a1.1* mutant shows a decreased expression of senescence-associated genes, such as *SAG12* and *SEN1*, and lacks SAVs in its mesophyll cells (Álvarez *et al*., 2010, 2012b). During senescence, autophagy is essential for maintaining viability via the effective recycling of nutrients (Bassham *et al*., 2006; Bassham, 2007; Xiong *et al*., 2007). Autophagy is a universal mechanism present in eukaryotic cells and consists of the degradation of cellular content to recycle nutrients, as well as to degrade damaged or toxic components. Autophagy is a constitutive process occurring at a basal level in growing cells, allowing for protein and organelle recycling. Plants induce autophagy under abiotic stress conditions, such as nutrient limitation or drug presence, and during normal development, the immune response and senescence. At the cellular level, autophagy is characterized by the *de novo* synthesis of double membrane- bound structures called autophagosomes, which engulf and deliver materials to the vacuole for breakdown (Nakatogawa *et al*., 2009). Autophagy-related (ATG) proteins are essential for the autophagy process and have been used to monitor autophagic activity in plants. The most commonly used protein is ATG8, which is covalently conjugated to phosphatidylethanolamine (PE) and tethered to the autophagosomes (Li and Vierstra, 2012; Pérez-Pérez *et al*., 2012).

The *des1* mutants accumulate ATG8, especially in its lipidated form, confirming that autophagy is induced in this mutant (Álvarez *et al.*, 2010, 2012b). Mutations in the *DES1* gene impede the degradation of cytosolic cysteine and, concomitantly, the generation of H_2S in the cytosol. H_2S is a known signalling molecule in animal systems. Recently, emerging experimental evidence has shown that H_2S is also a signalling molecule that is as important as NO and H_2O_2 in plant systems (Zhang *et al.*, 2008; García-Mata and Lamattina, 2010; Lisjak *et al.*, 2010; Zhang *et al.*, 2010; Chen *et al.*, 2011). The exogenous application of H_2S to *des1* mutants restores the differences that these mutants have with wild type plants at the phenotypic (the induced senescence is reverted), cellular (the SAVs and lipidated ATG8 are not over-accumulated) and transcriptomic (the transcriptome of *des1-1* plants that are treated with sulfide is very similar to that of the wild type plants) levels, demonstrating that sulfide negatively regulates autophagy in this model plant (Gotor *et al.*, 2013). In summary, DES1 and OAS-A1 modulate cysteine/sulfide homeostasis, which is critical for plant metabolism, development and stress responses in the cytosol (Romero *et al.*, 2013).

12.7 Conclusions

Our recent investigations of the OASTL family in *Arabidopsis* have uncovered functions for cysteine and its related molecules other than those of a mere metabolic role (see Fig. 12.4). Indeed, chloroplastic OAS can be used to form cysteine or S-Cys, which is a metabolite that is involved in the regulation and protection of the photosystems. In the mitochondria, cysteine maintains appropriate levels of cyanide, which regulates root hair development and modulates the plant immune response. Finally, cytosolic cysteine homeostasis orchestrates the plant response to pathogens, and cytosolic sulfide regulates autophagy. The mechanisms underlying these new signalling roles of cysteine and its related molecules are still unknown and constitute a novel and exciting field of research.

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References

Álvarez, C., Calo, L., Romero, L.C., García, I. and Gotor, C. (2010) An *O*-acetylserine(thiol)lyase homolog with L-cysteine desulfhydrase activity regulates cysteine homeostasis in *Arabidopsis*. *Plant Physiology* 152, 656–669.

Álvarez, C., Bermúdez, M.A., Romero, L.C., Gotor, C. and García, I. (2012a) Cysteine homeostasis plays an essential role in plant immunity. *New Phytologist* 193, 165–177.

Álvarez, C., García, I., Moreno, I., Pérez-Pérez, M.E., Crespo, J.L., Romero, L.C. and Gotor, C. (2012b) Cysteine-generated sulfide in the cytosol negatively regulates autophagy and modulates the transcriptional profile in *Arabidopsis*. *The Plant Cell* 24, 4621–4634.

Álvarez, C., García, I., Romero, L.C. and Gotor, C. (2012c) Mitochondrial sulfide detoxification requires a functional isoform *O*-acetylserine(thiol)lyase C in *Arabidopsis thaliana*. *Molecular Plant* 5, 1217–1226.

Barroso, C., Vega, J.M. and Gotor, C. (1995) A new member of the cytosolic *O*-acetylserine(thiol)lyase gene family in *Arabidopsis thaliana*. *FEBS Letters* 363, 1–5.

Barroso, C., Romero, L.C., Vega, J.M. and Gotor, C. (1997) Molecular characterization of the sulfur metabolism in plants. *Current Topics in Phytochemistry* 1, 19–29.

Bassham, D.C. (2007) Plant autophagy – more than a starvation response. *Current Opinion in Plant Biology* 10, 587–593.

Bassham, D.C., Laporte, M., Marty, F., Moriyasu, Y., Ohsumi, Y., Olsen, L.J. and Yoshimoto, K. (2006) Autophagy in development and stress responses of plants. *Autophagy* 2, 2–11.

Bermúdez, M.A., Paez-Ochoa, M.A., Gotor, C. and Romero, L.C. (2010) *Arabidopsis S*-sulfocysteine synthase activity is essential for chloroplast function and long-day light-dependent redox control. *The Plant Cell* 22, 403–416.

Bermúdez, M.A., Galmés, J., Moreno, I., Mullineaux, P.M., Gotor, C. and Romero, L.C. (2012) Photosynthetic adaptation to length of day is dependent on *S*-sulfocysteine synthase activity in the thylakoid. *Plant Physiology* 160, 274–288.

Bethke, P.C., Libourel, L.G.L., Reinohl, V. and Jones, R.L. (2006) Sodium nitroprusside, cyanide, nitrite, and nitrate break *Arabidopsis* seed dormancy in a nitric oxide-dependent manner. *Planta* 223, 805–812.

Bolchi, A., Petrucco, S., Tenca, P.L., Foroni, C. and Ottonello, S. (1999) Coordinate modulation of maize sulfate permease and ATP sulfurylase mRNAs in response to variations in sulfur nutritional status: stereospecific down-regulation by L-cysteine. *Plant Molecular Biology* 39, 527–537.

Bonner, E.R., Cahoon, R.E., Knapke, S.M. and Jez, J.M. (2005) Molecular basis of cysteine biosynthesis in plants – structural and functional analysis of *O*-acetylserine sulfhydrylase from *Arabidopsis thaliana*. *The Journal of Biological Chemistry* 280, 38803–38813.

Chen, J., Wu, F.H., Wang, W.H., Zheng, C.J., Lin, G.H., Dong, X.J., He, J.X., Pei, Z.M. and Zheng, H.L. (2011) Hydrogen sulphide enhances photosynthesis through promoting chloroplast biogenesis, photosynthetic enzyme expression, and thiol redox modification in *Spinacia oleracea* seedlings. *Journal of Experimental Botany* 62, 4481–4493.

Chivasa, S. and Carr, J.P. (1998) Cyanide restores *N* gene-mediated resistance to tobacco mosaic virus in transgenic tobacco expressing salicylic acid hydroxylase. *The Plant Cell* 10, 1489–1498.

Cohn, M.A. and Hughes, J.A. (1986) Seed dormancy in red rice. 5. Response to azide, hydroxylamine and cyanide. *Plant Physiology* 80, 531–533.

Dietz, K.J. and Pfannschmidt, T. (2011) Novel regulators in photosynthetic redox control of plant metabolism and gene expression. *Plant Physiology* 155, 1477–1485.

Dominguez-Solis, J.R., Gutierrez-Alcala, G., Vega, J.M., Romero, L.C. and Gotor, C. (2001) The cytosolic *O*-acetylserine(thiol)lyase gene is regulated by heavy metals and can function in cadmium tolerance. *The Journal of Biological Chemistry* 276, 9297–9302.

Dominguez-Solis, J.R., López-Martín, M.C., Ager, F.J., Ynsa, M.D., Romero, L.C. and Gotor, C. (2004) Increased cysteine availability is essential for cadmium tolerance and accumulation in *Arabidopsis thaliana*. *Plant Biotechnology Journal* 2, 469–476.

Donato, D.B., Nichols, O., Possingham, H., Moore, M., Ricci, P.F. and Noller, B.N. (2007) A critical review of the effects of gold cyanide-bearing tailings solutions on wildlife. *Environment International* 33, 974–984.

Droux, M. (2004) Sulfur assimilation and the role of sulfur in plant metabolism: a survey. *Photosynthesis Research* 79, 331–348.

Droux, M., Ruffet, M.L., Douce, R. and Job, D. (1998) Interactions between serine acetyltransferase and *O*-acetylserine (thiol) lyase in higher plants – structural and kinetic properties of the free and bound enzymes. *European Journal of Biochemistry* 255, 235–245.

García, I., Castellano, J.M., Vioque, B., Solano, R., Gotor, C. and Romero L.C. (2010) Mitochondrial β-cyanoalanine synthase is essential for root hair formation in *Arabidopsis thaliana*. *The Plant Cell* 22, 3268–3279.

García, I., Rosas, T., Bejarano, E.R., Gotor, C. and Romero, L.C. (2013) Transient transcriptional regulation of the *CYS-C1* gene and cyanide accumulation upon pathogen infection in the plant immune response. *Plant Physiology* 162, 2015–2027.

García-Mata, C. and Lamattina, L. (2010) Hydrogen sulphide, a novel gasotransmitter involved in guard cell signalling. *New Phytologist* 188, 977–984.

Glawischnig, E. (2007) Camalexin. *Phytochemistry* 68, 401–406.

Gotor, C. and Romero, L.C. (2013) *S*-sulfocysteine synthase function in sensing chloroplast redox status. *Plant Signaling and Behavior* 8(3): e23313.

Gotor, C., Álvarez, C., Bermúdez, M.A., Moreno, I., García, I. and Romero, L.C. (2010) Low abundance does not mean less importance in cysteine metabolism. *Plant Signaling and Behavior* 5, 1028–1030.

Gotor, C., García, I., Crespo, J.L. and Romero, L.C. (2013) Sulfide as a signaling molecule in autophagy. *Autophagy* 9, 609–611.

Gutierrez-Marcos, J.F., Roberts, M.A., Campbell, E.I. and Wray, J.L. (1996) Three members of a novel small gene-family from *Arabidopsis thaliana* able to complement functionally an *Escherichia coli* mutant defective in PAPS reductase activity encode proteins with a thioredoxin-like domain and "APS reductase" activity. *Proceedings National Academy Sciences of the United States of America* 93, 13377–13382.

Haas, F.H., Heeg, C., Queiroz, R., Bauer, A., Wirtz, M. and Hell, R. (2008) Mitochondrial serine acetyltransferase functions as a pacemaker of cysteine synthesis in plant cells. *Plant Physiology* 148, 1055–1067.

Hatzfeld, Y., Lee, S., Lee, M., Leustek, T. and Saito, K. (2000) Functional characterization of a gene encoding a fourth ATP sulfurylase isoform from *Arabidopsis thaliana*. *Gene* 248, 51–58.

Hawkesford, M.J. and De Kok, L.J. (2006) Managing sulphur metabolism in plants. *Plant, Cell and Environment* 29, 382–395.
Heath, M.C. (2000) Hypersensitive response-related death. *Plant Molecular Biology* 44, 321–334.
Heeg, C., Muller, C., Throm, C., Rippe, K., Rybin, V., Wirtz, M. and Hell, R. (2007) Protein–protein interactions define regulatory properties of the cysteine synthase complex. *FEBS Journal* 274, 241–241.
Heeg, C., Kruse, C., Jost, R., Gutensohn, M., Ruppert, T., Wirtz, M. and Hell, R. (2008) Analysis of the *Arabidopsis O*-acetylserine(thiol)lyase gene family demonstrates compartment-specific differences in the regulation of cysteine synthesis. *The Plant Cell* 20, 168–185.
Heldt, W.H., Werdan, K., Milovancev, M. and Geller, G. (1973) Alkalization of the chloroplast stroma caused by light-dependent proton flux into the thylakoid space. *Biochimica and Biophysica Acta* 314, 224–241.
Hell, R. (1997) Molecular physiology of plant sulfur metabolism. *Planta* 202, 138–148.
Hirai, M.Y. and Saito, K. (2008) Analysis of systemic sulfur metabolism in plants using integrated '-omics' strategies. *Molecular BioSystems* 4, 967–973.
Howarth, J.R., Dominguez-Solis, J.R., Gutierrez-Alcala, G., Wray, J.L., Romero, L.C. and Gotor, C. (2003a) The serine acetyltransferase gene family in *Arabidopsis thaliana* and the regulation of its expression by cadmium. *Plant Molecular Biology* 51, 589–598.
Howarth, J.R., Fourcroy, P., Davidian, J.C., Smith, F.W. and Hawkesford, M.J. (2003b) Cloning of two contrasting high-affinity sulfate transporters from tomato induced by low sulfate and infection by the vascular pathogen *Verticillium dahliae*. *Planta* 218, 58–64.
Jacob, C., Giles, G.I., Giles, N.M. and Sies, H. (2003) Sulfur and selenium: the role of oxidation state in protein structure and function. *Angewandte Chemie* 42, 4742–4758.
Jost, R., Berkowitz, O., Wirtz, M., Hopkins, L., Hawkesford, M.J. and Hell, R. (2000) Genomic and functional characterization of the *oas* gene family encoding *O*-acetylserine (thiol) lyases, enzymes catalyzing the final step in cysteine biosynthesis in *Arabidopsis thaliana*. *Gene* 253, 237–247.
Kabil, O. and Banerjee, R. (2010) Redox biochemistry of hydrogen sulfide. *The Journal of Biological Chemistry* 285, 21903–21907.
Khan, M.S., Haas, F.H., Samami, A.A., Gholami, A.M., Bauer, A., Fellenberg, K., Reichelt, M., Hansch, R., Mendel, R.R., Meyer, A.J., Wirtz, M. and Hell, R. (2010) Sulfite reductase defines a newly discovered bottleneck for assimilatory sulfate reduction and is essential for growth and development in *Arabidopsis thaliana*. *The Plant Cell* 22, 1216–1231.
Krueger, S., Niehl, A., Lopez Martin, M.C., Steinhauser, D., Donath, A., Hildebrandt, T., Romero, L.C., Hoefgen, R., Gotor, C. and Hesse, H. (2009) Analysis of cytosolic and plastidic serine acetyltransferase mutants and subcellular metabolite distributions suggests interplay of the cellular compartments for cysteine biosynthesis in *Arabidopsis*. *Plant Cell and Environment* 32, 349–367.
Kumaran, S., Yi, H., Krishnan, H.B. and Jez, J.M. (2009) Assembly of the cysteine synthase complex and the regulatory role of protein–protein interactions. *The Journal of Biological Chemistry* 284, 10268–10275.
Lamb, C. and Dixon, R.A. (1997) The oxidative burst in plant disease resistance. *Annual Review of Plant Physiology and Plant Molecular Biology* 48, 251–275.
Lee, B.-R., Koprivova, A. and Kopriva, S. (2011) The key enzyme of sulfate assimilation, adenosine 5′-phosphosulfate reductase, is regulated by HY5 in *Arabidopsis*. *The Plant Journal* 67, 1042–1054.
Lee, M. and Leustek, T. (1999) Identification of the gene encoding homoserine kinase from *Arabidopsis thaliana* and characterization of the recombinant enzyme derived from the gene. *Archives of Biochemistry and Biophysics* 372, 135–142.
León, J., Romero, L.C., Galván, F. and Vega, J.M. (1987) Purification and physicochemical characterization of *O*-acetyl-l-serine sulfhydrilase from *Chlamydomonas reinhardtii*. *Plant Science* 53, 93–99.
Leustek, T., Martin, M.N., Bick, J.A. and Davies, J.P. (2000) Pathways and regulation of sulfur metabolism revealed through molecular and genetic studies. *Annual Review of Plant Physiology and Plant Molecular Biology* 51, 141–165.
Li, F. and Vierstra, R.D. (2012) Autophagy: a multifaceted intracellular system for bulk and selective recycling. *Trends in Plant Sciences* 17, 526–537.
Lisjak, M., Srivastava, N., Teklic, T., Civale, L., Lewandowski, K., Wilson, I., Wood, M.E., Whiteman, M. and Hancock, J.T. (2010) A novel hydrogen sulfide donor causes stomatal opening and reduces nitric oxide accumulation. *Plant Physiology and Biochemistry* 48, 931–935.
López-Martín, M.C., Becana, M., Romero, L.C. and Gotor, C. (2008a) Knocking out cytosolic cysteine synthesis compromises the antioxidant capacity of the cytosol to maintain discrete concentrations of hydrogen peroxide in *Arabidopsis*. *Plant Physiology* 147, 562–572.

López-Martín, M.C., Romero, L.C. and Gotor, C. (2008b) Cytosolic cysteine in redox signaling. *Plant Signaling and Behavior* 3, 880–881.

Lunn, J.E., Droux, M., Martin, J. and Douce, R. (1990) Localization of ATP sulfurylase and *O*-acetylserine (thiol)lyase in spinach leaves. *Plant Physiology* 94, 1345–1352.

Moriarty, R.M., Naithani, R. and Surve, B. (2007) Organosulfur compounds in cancer chemoprevention. *Mini Reviews in Medicinal Chemistry* 7, 827–838.

Mugford, S.G., Yoshimoto, N., Reichelt, M., Wirtz, M., Hill, L., Mugford, S.T., Nakazato, Y., Noji, M., Takahashi, H., Kramell, R., Gigolashvili, T., Flugge, U.I. *et al*. (2009) Disruption of adenosine-5′-phosphosulfate kinase in *Arabidopsis* reduces levels of sulfated secondary metabolites. *The Plant Cell* 21, 910–927.

Nakamura, T., Iwahashi, H. and Eguchi, Y. (1984) Enzymatic proof for the identity of the *S*-sulfocysteine synthase and cysteine synthase B of *Salmonella typhimurium*. *Journal of Bacteriology* 158, 1122–1127.

Nakatogawa, H., Suzuki, K., Kamada, Y. and Ohsumi, Y. (2009) Dynamics and diversity in autophagy mechanisms: lessons from yeast. *Nature Reviews Molecular Cell Biology* 10, 458–467.

Noji, M., Inoue, K., Kimura, N., Gouda, A. and Saito, K. (1998) Isoform-dependent differences in feedback regulation and subcellular localization of serine acetyltransferase involved in cysteine biosynthesis from *Arabidopsis thaliana*. *The Journal of Biological Chemistry* 273, 32739–32745.

Noji, M., Goulart Kawashima, C., Obayashi, T. and Saito, K. (2006) *In silico* assessment of gene function involved in cysteine biosynthesis in *Arabidopsis*: expression analysis of multiple isoforms of serine acetyltransferase. *Amino Acids* 30, 163–171.

Park, S. and Imlay, J.A. (2003) High levels of intracellular cysteine promote oxidative DNA damage by driving the Fenton reaction. *Journal of Bacteriology* 185, 1942–1950.

Pérez-Pérez, M.E., Lemaire, S.D. and Crespo, J.L. (2012) Reactive oxygen species and autophagy in plants and algae. *Plant Physiology* 160, 156–164.

Piotrowski, M. (2008) Primary or secondary? Versatile nitrilases in plant metabolism. *Phytochemistry* 69, 2655–2667.

Rausch, T. and Wachter, A. (2005) Sulfur metabolism: a versatile platform for launching defence operations. *Trends in Plant Science* 10, 503–509.

Romero, L.C., Dominguez-Solis, J.R., Gutierrez-Alcala, G. and Gotor, C. (2001) Salt regulation of *O*-acetylserine(thiol)lyase in *Arabidopsis thaliana* and increased tolerance in yeast. *Plant Physiology and Biochemistry* 39, 643–647.

Romero, L.C., García, I. and Gotor, C. (2013) L-Cysteine desulfhydrase 1 modulates the generation of the signaling molecule sulfide in plant cytosol. *Plant Signaling and Behavior* 8(5): e24007.

Rouached, H., Berthomieu, P., El Kassis, E., Cathala, N., Catherinot, V., Labesse, G., Davidian, J.C. and Fourcroy, P. (2005) Structural and functional analysis of the C-terminal STAS (sulfate transporter and anti-sigma antagonist) domain of the *Arabidopsis thaliana* sulfate transporter SULTR1.2. *The Journal of Biological Chemistry* 280, 15976–15983.

Saito, K. (2004) Sulfur assimilatory metabolism. The long and smelling road. *Plant Physiology* 136, 2443–2450.

Seo, S., Mitsuhara, I., Feng, J., Iwai, T., Hasegawa, M. and Ohashi, Y. (2011) Cyanide, a coproduct of plant hormone ethylene biosynthesis, contributes to the resistance of rice to blast fungus. *Plant Physiology* 155, 502–514.

Setya, A., Murillo, M. and Leustek, T. (1996) Sulfate reduction in higher plants: molecular evidence for a novel 5′-adenylylsulfate reductase. *Proceedings of the National Academy of Sciences of the United States of America* 93, 13383–13388.

Shibagaki, N. and Grossman, A.R. (2004) Probing the function of STAS domains of the *Arabidopsis* sulfate transporters. *The Journal of Biological Chemistry* 279, 30791–30799.

Siegien, I. and Bogatek, R. (2006) Cyanide action in plants – from toxic to regulatory. *Acta Physiologiae Plantarum* 28, 483–497.

Takahashi, H., Kopriva, S., Giordano, M., Saito, H. and Hell, R. (2011) Sulfur assimilation in photosynthetic organisms: molecular functions and regulations of transporters and assimilatory enzymes. *Annual Review of Plant Biology* 62, 157–184.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011) mega5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28, 2731–2739.

Vauclare, P., Kopriva, S., Fell, D., Suter, M., Sticher, L., von Ballmoos, P., Krahenbuhl, U., den Camp, R.O. and Brunold, C. (2002) Flux control of sulphate assimilation in *Arabidopsis thaliana*: adenosine 5′-phosphosulphate reductase is more susceptible than ATP sulphurylase to negative control by thiols. *Plant Journal* 31, 729–740.

Vidmar, J.J., Tagmount, A., Cathala, N., Touraine, B. and Davidian, J.E. (2000) Cloning and characterization of a root specific high-affinity sulfate transporter from *Arabidopsis thaliana*. *FEBS Letters* 475, 65–69.

Vlot, A.C., Klessig, D.F. and Park, S.W. (2008) Systemic acquired resistance: the elusive signal(s). *Current Opinion in Plant Biology* 11, 436–442.

Watanabe, M., Kusano, M., Oikawa, A., Fukushima, A., Noji, M. and Saito, K. (2008) Physiological roles of the β-substituted alanine synthase gene family in *Arabidopsis*. *Plant Physiology* 146, 310–320.

Wirtz, M. and Droux, M. (2005) Synthesis of the sulfur amino acids: cysteine and methionine. *Photosynthesis Research* 86, 345–362.

Wirtz, M. and Hell R. (2006) Functional analysis of the cysteine synthase protein complex from plants: structural, biochemical and regulatory properties. *Journal of Plant Physiology* 163, 273–286.

Wong, C.E., Carson, R.A. and Carr, J.P. (2002) Chemically induced virus resistance in *Arabidopsis thaliana* is independent of pathogenesis-related protein expression and the *NPR1* gene. *Molecular Plant Microbe Interactions* 15, 75–81.

Wu, W. and Berkowitz, G.A. (1992) Stromal pH and photosynthesis are affected by electroneutral K and H exchange through chloroplast envelope ion channels. *Plant Physiology* 98, 666–672.

Xiong, Y., Contento, A.L., Nguyen, P.Q. and Bassham, D.C. (2007) Degradation of oxidized proteins by autophagy during oxidative stress in *Arabidopsis*. *Plant Physiology* 143, 291–299.

Yamaguchi, Y., Nakamura, T., Kusano, T. and Sano, H. (2000) Three *Arabidopsis* genes encoding proteins with differential activities for cysteine synthase and β-cyanoalanine synthase. *Plant and Cell Physiology* 41, 465–476.

Yonekura-Sakakibara, K., Onda, Y., Ashikari, T., Tanaka, Y., Kusumi, T. and Hase, T. (2000) Analysis of reductant supply systems for ferredoxin-dependent sulfite reductase in photosynthetic and nonphotosynthetic organs of maize. *Plant Physiology* 122, 887–894.

Zhang, H., Hu, L.Y., Hu, K.D., He, Y.D., Wang, S.H. and Luo, J.P. (2008) Hydrogen sulfide promotes wheat seed germination and alleviates oxidative damage against copper stress. *Journal of Integrative Plant Biology* 50, 1518–1529.

Zhang, H., Tan, Z.Q., Hu, L.Y., Wang, S.H., Luo, J.P. and Jones, R.L. (2010) Hydrogen sulfide alleviates aluminum toxicity in germinating wheat seedlings. *Journal of Integrative Plant Biology* 52, 556–567.